

# Different Oat Ingredients Stimulate Specific Microbial Metabolites in the Gut Microbiome of Three Human Individuals in Vitro

Pieter Van den Abbeele,<sup>†</sup> Alison Kamil,<sup>‡</sup> Lisa Fleige,<sup>‡</sup> Yongsoo Chung,<sup>‡</sup> Peter De Chavez,<sup>‡</sup> and Massimo Marzorati<sup>\*,†,§</sup>

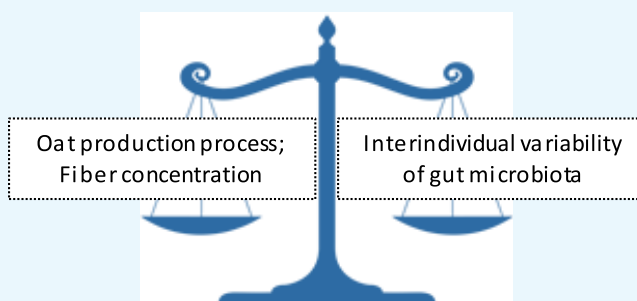
<sup>†</sup>ProDigest bvba, Technologiepark 3, 9052 Ghent, Belgium

<sup>‡</sup>PepsiCo, Inc. R&D Nutrition Sciences, 617 W Main Street, Barrington, Illinois 60010, United States

<sup>§</sup>Center of Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium

## S Supporting Information

**ABSTRACT:** We used a standardized in vitro simulation of the intestinal environment of three human donors to investigate the effect of six oat ingredients, which were produced by the application of different processing techniques, on the gut microbial community. Fructooligosaccharide was used as the positive control. Consistent changes in pH and gas production, on average  $-0.4$  pH units and  $+32$  kPa, indicated the high fermentability of the oat ingredients, and the resulting increased production of metabolites that are considered as beneficial for human health. These metabolites included acetate and lactate, but mostly propionate ( $+13.6$  mM on average). All oat ingredients resulted in increased bifidobacteria levels with an average increase of  $0.73$  log. Moreover, a decreased production of proteolytic markers was observed, including branched short-chain fatty acids and ammonium. The results were donor-specific and product-specific. The results suggested an association between the total amounts of dietary fiber and the prebiotic potentials of different ingredients. Furthermore, as mechanical processing of oat products has previously been linked to increased extractability of dietary fibers, the obtained results suggest that different processing techniques might have impacted the potential functional properties of the final ingredients.



## Prebiotic effect of oat ingredients

## INTRODUCTION

In the past decade, study findings have revealed associations between the intestinal microbial community and human health and disease. The gut microbiota affects the immune system of the host,<sup>1</sup> participates in fat storage and synthesis in the body,<sup>2,3</sup> transforms phytoestrogens into potent estrogenic metabolites,<sup>4</sup> and reduces the risk of colon cancer.<sup>5,6</sup> Modulation of the human gut microbiota has potential as a strategy for improving human health. Changes can be accomplished using supplementation of prebiotics. These nondigestible substrates are selectively used by the host micro-organisms conferring a health benefit.<sup>7</sup> Prebiotic use can have various beneficial effects on the human body (e.g., improved intestinal barrier function, reduction of intestinal inflammation, modulation of immune status, and reduced risk of obesity, colon cancer, and type II diabetes).<sup>8</sup>

While inulin-type fructooligosaccharides (FOSs) are one of the most studied prebiotic compounds,<sup>9,10</sup> several novel classes of prebiotics are emerging. For example, studies have revealed some health-promoting effects of the oat fiber consumed during oat product intake. The associated effects include cholesterol reduction,<sup>11–13</sup> modulation of blood glucose

levels,<sup>13–15</sup> improved weight management,<sup>16</sup> and immune regulation.<sup>17,18</sup>

Many of these effects have been attributed to the  $\beta$ -glucans, which is a group of soluble fibers present in oats. These non-starch polysaccharides consist of  $\beta$ -linked chains of D-glucose monomers. Depending on the source, each  $\beta$ -glucan can have a different branching structure, viscosity, solubility, and molecular weight; these characteristics affect the functional properties of  $\beta$ -glucans.<sup>16</sup> Oat typically contains a mixture of unbranched  $\beta$ -glucans with  $\beta$ -1,3- and  $\beta$ -1,4-glycosidic linkages.<sup>16</sup> Variations in the processing conditions of oats have direct effects on  $\beta$ -glucan release from the cell wall of the oat groats. The resulting structural differences account for the differences in physiological properties and related health effects of oat  $\beta$ -glucans. Mechanical processing increases the release of  $\beta$ -glucans from the oat groats by reducing the particle size,<sup>19</sup> and hydrothermal processing reduces the extractability of oat  $\beta$ -glucans.<sup>19,20</sup> Hydrothermal processing increases oat product

Received: June 17, 2018

Accepted: September 17, 2018

Published: October 2, 2018

viscosity,<sup>20</sup> which can reduce glucose and cholesterol absorption in the gastrointestinal tract.<sup>21,22</sup>

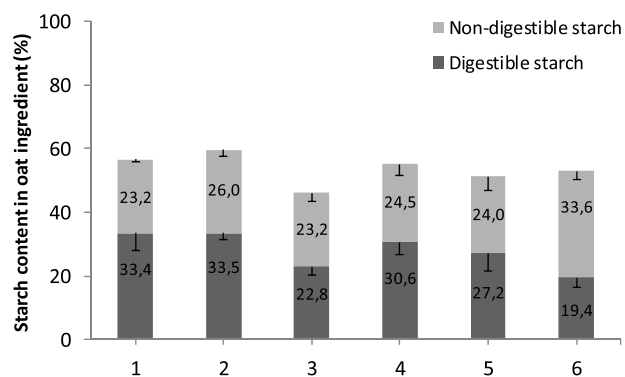
Although study findings have revealed oat  $\beta$ -glucan structural differences and some associations with health and disease, the links between oats processed using different methods and the resulting potential prebiotic properties of oat  $\beta$ -glucans on the gut microbiome remain poorly understood.

In vitro approaches are widely used to study the possible prebiotic properties of selected food ingredients. A wide range of in vitro systems are available. These range from short-term colonic batch incubation<sup>23–25</sup> to long-term continuous models that allow in-depth study of intestinal microbial processes when test compounds are added under representative environmental conditions.<sup>26–28</sup> Because environmental factors can be strictly controlled, in vitro studies are an appropriate alternative to in vivo studies for providing mechanistic insights into the interactions between food ingredients and effects on the human gut microbiome. To obtain representative microbial communities, in vitro gut models rely on fecal samples from human subjects.<sup>23,29</sup> There are large differences in gut microbial composition between human individuals.<sup>30</sup> These differences can affect the utilization of dietary ingredients via different metabolic pathways.<sup>31,32</sup> Therefore, it is important to account for interindividual variability in gut microbial composition during in vivo and in vitro studies.

Our main study objective was to investigate the potential prebiotic effects of supplementation of six types of oat ingredients on the colonic microbiota of three individuals upon digestion. The oat ingredients were produced using different processing techniques. We used FOS as the positive control supplement. We used a standardized in vitro simulation of the intestinal environment to obtain mechanistic insights into the microbial activity and composition changes that occurred in response to the fermentation of different oat ingredients.

## RESULTS

**Digestibility of Starch from Oat Ingredients.** The fractions of digestible and nondigestible starch were quantified by determining the starch levels in the initial oat ingredients and at the end of the small intestinal incubation (Figure 1). A



**Figure 1.** Digestion and absorption of starch from oat ingredients. Average total starch levels (%) of different oat ingredients (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked oat flour; 6 = pre-cooked Morrison oat flour), separated into digestible (and subsequently absorbed) and non-digestible starch fractions (%), as quantified at the end of the small intestinal incubation ( $\pm$ standard deviation).

significant part of the starch was removed for each of the products during upper gastrointestinal digestion and absorption. For old-fashioned oats (1), instant oat flakes (2), and steel-cut oats (4), >30% of the product was removed via starch digestion and subsequent absorption. These changes corresponded to the digestion and absorption of starch of >55% (Table 1).

**Overall Microbial Metabolic Activity in Terms of Acidification and Gas Production.** The overall degrees of acidification and gas production were markers for the intensity of bacterial metabolism of the test compounds (Table 2; Supporting Information Table 2). The positive control (FOS) resulted in the greatest pH decrease ( $-1.05$ ) and gas production ( $+50.8$  kPa) during the 0–48 h of incubation period. Microbial fermentation was less pronounced during the incubation of the blank negative control (BNC ( $-0.05$  and  $+20.9$  kPa)). A pH increase ( $+0.14$ ) rather than a decrease occurred in the FOS samples during the 24–48 h time interval, probably by depletion of the test ingredient.

Compared with BNC, there was a statistically significant increase in colonic gas production and decrease in colonic pH for all oat ingredients. However, these changes were less than the changes in the FOS samples. Most of the effects of the oat ingredients on these parameters occurred during the first 24 h of incubation with (i) a decrease in the mean pH of approximately  $-0.40$  (versus  $-0.04$  for BNC), and (ii) an increase in gas pressure varying between  $+29$  and  $+35$  kPa, depending on the test ingredient (versus  $\pm 18$  kPa for BNC). There were no statistically significant differences between the oat ingredients.

There were specific inter-donor differences in gas production (Supporting Information Table 3). Overall, gas production during the first 6 h of incubation was greater for donor A, compared with donor C and especially donor B.

**Microbial Metabolic Activity in Terms of Short-Chain Fatty Acids (SCFAs), Lactate (LA), and  $\text{NH}_4^+$  Production.** Acetate, propionate, butyrate, and lactate are metabolites that result from microbial carbohydrate metabolism (Figure 2; Supporting Information Table 4). Final acetate levels were the lowest in the control samples (BNC; 14.8 mM); the FOS samples had the highest acetate levels (35.1 mM) (Figure 2A). All oat ingredient samples led to a statistically significant increase in acetate levels relative to the BNC samples. However, the increase was less than that for the FOS samples. Most of the acetate production occurred during the 0–24 h interval. However, statistically significant amounts of acetate were produced between 24 and 48 h for several oat ingredients, compared with the FOS and BNC samples (i.e., oat bran (3), steel-cut oats (4), pre-cooked oat flour (5), and pre-cooked Morrison oat flour (6)).

Control sample incubation resulted in the lowest propionate levels for the three donors tested (Figure 2B). For all donors, the increase in propionate production was greater for the six oat ingredients versus the positive control FOS; the oat bran (3) samples had the highest propionate production (14.3 mM). There were specific inter-donor differences in initial propionate production. During the first 6 h of incubation, donor A had higher propionate levels, compared with donor C and especially donor B (Supporting Information Table 3).

The highest butyrate production occurred in the FOS samples. This difference was especially attributed to a high production during the final 24 h of incubation (3.7 mM). Compared with the BNC samples, none of the oat ingredient

**Table 1. Processing Techniques, Cooking Procedures (with the Moisture Content (%) Determined by Gravimetric Measurement),  $\beta$ -Glucan Content (% Determined According to OAC Official Method 995.16), and the Total Dietary Fiber Content (% Determined According to OAC Official Method 991.43) for the Oat Ingredients That Were Evaluated**

name	processing techniques				cooking (moisture content after cooking (%))	$\beta$ -glucan (% dry wt)	total dietary fiber (% dry wt)
	cutting	steaming	pressing	grinding			
1 old-fashioned oats	–	+	+	–	5 min boiling (74.8%)	4.83	9.7
2 instant oat flakes	+	+	+	–	1 min boiling (81.0%)	5.02	8.8
3 oat bran	+	+	+	+	1 min boiling (82.8%)	7.62	14.8
4 steel-cut oats	+	–	–	–	30 min simmering (72.1%)	4.94	7.9
5 pre-cooked oat flour	–	+	–	++	pre-cooked (9.1%)	3.94	9.7
6 pre-cooked Morrison oat flour	–	+	–	++	pre-cooked (10.0%)	6.31	11.6

samples had significantly higher butyrate production (Figure 2C). Amongst the oat ingredients, steel-cut oats (4) resulted in the highest butyrate production levels (3.1 mM).

Lactate levels (Figure 2D) were low in the control incubation (1.3 mM). The FOS samples had the highest mean lactate production levels during the 0–24 h time interval (5.9 mM), and high consumption during the final 24 h of incubation (–3.8 mM). The consumption of lactate was lower (i.e., between –0.6 and –1.1 mM) for the oat ingredient samples. There were significant correlations ( $R^2 = 0.83$ ;  $p < 0.0001$ ) between lactate consumption during the last 24 h of incubation and butyrate production during the same time interval for FOS and the six oat ingredients (Figure 3).

Branched SCFAs and ammonium are markers of microbial protein metabolism. Branched SCFA production was strongly decreased for the FOS and the six oat ingredient samples compared to the BNC (Table 3; Supporting Information Table S5). The FOS samples had the greatest decline in branched SCFA production (i.e., 0.2 mM for FOS versus 2.1 mM for BNC). The ammonium concentration was strongly decreased by all oat ingredients, compared with BNC (Table 3). For the branched SCFAs, an even lower production of ammonium was associated with the fermentation of FOS. Among the oat ingredients, the lowest production of branched SCFAs and ammonium occurred for oat bran (3). The pre-cooked oat flour (5) samples had the highest branched SCFA production and steel-cut oats (4) samples had even higher ammonium production.

#### Changes in Microbial Community Composition.

Quantitative polymerase chain reaction (qPCR) analysis was performed to assess the effects of the test substances on specific taxonomic groups of bacteria (Bifidobacteria, Lactobacilli, and Firmicutes) (Table 4). Firmicutes levels remained stable throughout colonic incubation. The different test substances had no effects on the concentration of bacteria belonging to this phylum.

The use of FOS resulted in the greatest increase in *Bifidobacterium* spp. levels (+1.03 log). Compared with the BNC, the use of every oat ingredient resulted in significantly increased *Bifidobacterium* spp. levels, but the changes were less than when FOS was used. There were no statistically significant differences between the different oat ingredients.

*Lactobacillus* spp. levels were characterized by distinct inter-donor differences. For donor A, none of the test substances had a significant effect on *Lactobacillus* spp. levels. Strong increases were obtained for donor B, because *Lactobacillus* spp. was under-represented in the fecal inoculum of this donor. The increase was greatest after FOS supplementation (+3.5 log),

but the use of oat ingredients also resulted in increased *Lactobacillus* spp. levels that were mostly similar to the BNC. Compared with the BNC, the use of pre-cooked oat flour resulted in a small additional increase in *Lactobacillus* levels (+0.3 log). For donor C, there was a strong effect on *Lactobacillus* spp. levels for the positive control FOS samples. The use of the oat ingredients did not result in any statistically significant effects on *Lactobacilli*.

**Principal Component Analysis (PCA).** To compare the final effects among the test products, the increase and decrease from 0 to 48 h of incubation for all parameters were used to create a PCA biplot (Figure 4). The only exception was lactate; this intermediate metabolite is produced and subsequently consumed and peak levels occur at 24 h. Therefore, the use of levels at 48 h is not valid for the interpretation of overall effects. The results of the analysis indicated that the positive (FOS) and negative (BNC) control samples grouped into separate clusters. For the oat ingredients, the clusters were mainly associated with inter-donor differences. Within these donor-associated clusters, old-fashioned oats (1), instant oat flakes (2), pre-cooked oat flour (5) and pre-cooked Morrison oat flour (6) clustered closely together, while oat bran (3) and steel-cut oat (4) samples tended to be more different from the other oat ingredients (except for steel-cut oats (4) in donor A). Oat bran (3) samples tended to be more similar to the FOS samples, while steel-cut oat (4) samples trended towards the negative control samples.

## DISCUSSION

We used standardized in vitro simulation of the intestinal environment and FOS as a positive control to investigate the fermentability of six oat ingredients. Oat products contain a large fraction of digestible starch. Because this starch is absorbed at the level of the small intestine after conversion into maltose and glucose, an in vitro simulation of small intestinal absorption preceded the colonic incubations. To validate this dialysis procedure, the digestion and subsequent absorption characteristics of the oat ingredients were determined. Consistent with Kim et al.'s finding, approximately 50% of the starch in different cooked oat ingredients was digested and absorbed.<sup>37</sup> The subsequent colonic fermentation elicited consistent changes at the metabolic activity level (i.e., in SCFA, lactic acid, and  $\text{NH}_4^+$  production), with donor-specific differences, probably partly influenced by the remaining indigestible starch content of the oat ingredients.

The consistent changes in pH and gas production indicated the high fermentability of the oat ingredients. This fermentation resulted in increased numbers of *Bifidobacteria*.



Table 2. Overall Metabolic Activity (Acidification and Gas Production)<sup>a</sup>

	time (h)	donor A/B/C						
		1	2	3	4	5	6	7
pH	Δ0–24	−0.42 <sup>a</sup> ± 0.04	−0.41 <sup>a</sup> ± 0.07	−0.38 <sup>a</sup> ± 0.07	−0.36 <sup>a</sup> ± 0.05	−0.43 <sup>a</sup> ± 0.03	−0.40 <sup>a</sup> ± 0.06	−1.19 <sup>b</sup> ± 0.26
	Δ24–48	0.01 <sup>a</sup> ± 0.06	0.01 <sup>a</sup> ± 0.06	−0.01 <sup>a</sup> ± 0.05	0.01 <sup>a</sup> ± 0.04	0.03 <sup>a</sup> ± 0.07	0.02 <sup>a</sup> ± 0.07	0.14 <sup>b</sup> ± 0.15
	Δ0–48	−0.41 <sup>a</sup> ± 0.09	−0.39 <sup>a</sup> ± 0.10	−0.39 <sup>a</sup> ± 0.08	−0.36 <sup>a</sup> ± 0.06	−0.40 <sup>a</sup> ± 0.09	−0.39 <sup>a</sup> ± 0.08	−1.05 <sup>b</sup> ± 0.35
gas pressure (pK <sub>a</sub> )	Δ0–24	34.3 <sup>a</sup> ± 8.7	32.7 <sup>a</sup> ± 7.2	29.8 <sup>a</sup> ± 6.0	29.8 <sup>a</sup> ± 5.4	34.5 <sup>a</sup> ± 8.3	31.5 <sup>a</sup> ± 6.0	49.8 <sup>b</sup> ± 14.1
	Δ24–48	0.0 ± 4.6	1.0 ± 3.6	4.4 ± 1.3	2.7 ± 1.0	0.4 ± 4.4	−1.4 ± 9.7	1.0 ± 11.1
	Δ0–48	34.3 <sup>a</sup> ± 4.5	33.6 <sup>a</sup> ± 4.1	34.2 <sup>a</sup> ± 6.6	32.5 <sup>a</sup> ± 5.2	35.0 <sup>a</sup> ± 4.3	30.1 <sup>a</sup> ± 9.0	50.8 <sup>b</sup> ± 9.0
								18.2 <sup>c</sup> ± 2.6
								2.7 ± 1.4
								20.9 <sup>c</sup> ± 3.2

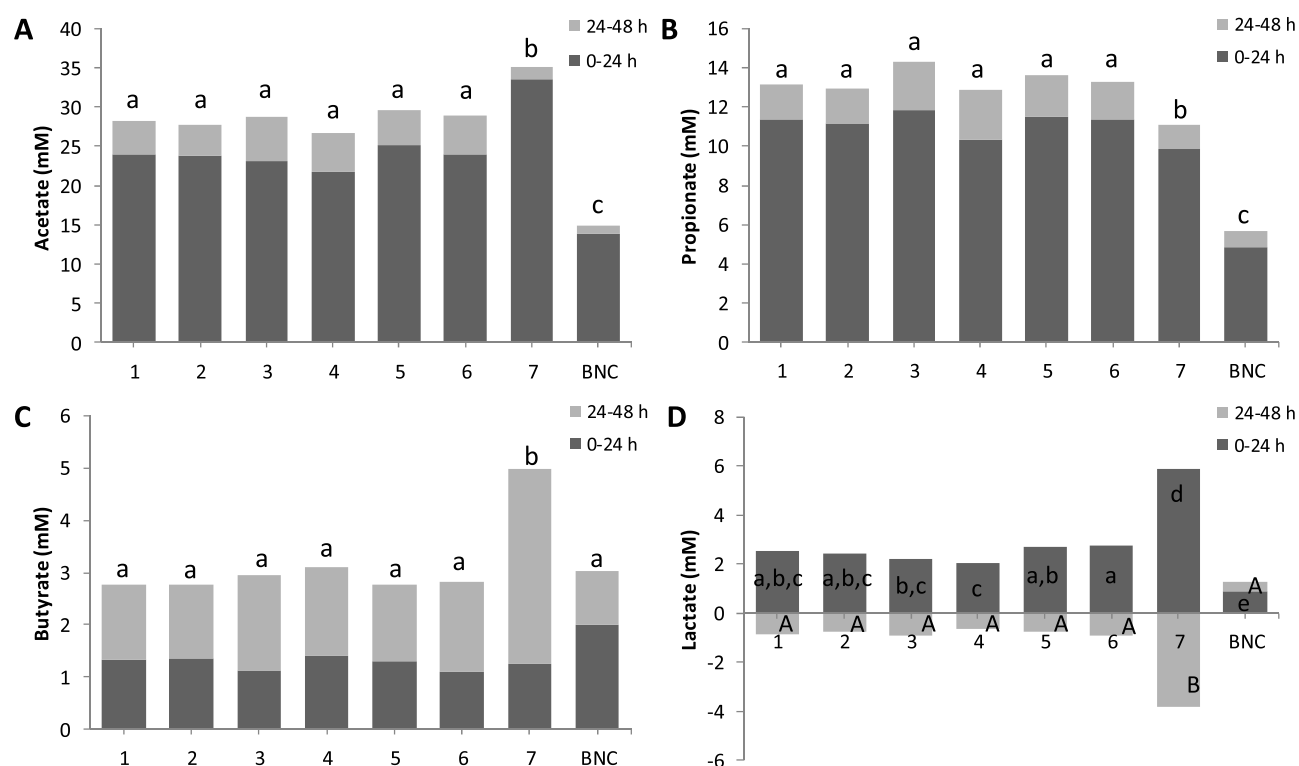
<sup>a</sup>Mean (±standard deviation) values for pH changes and increases in gas pressure (kPa) between 0–24, 24–48, and 0–48 h, upon fermentation of the different test compounds (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked Morrison oat flour; 6 = pre-cooked Morrison oat flour; 7 = fructooligosaccharide) by human fecal microbiota of three different donors (A, B, and C), versus their respective blank negative controls (BNCs). For optimal observation of consistent effects over the microbiota of the three donors, the mean values ("donor A/B/C") are presented ( $n = 9$ ;  $n = 3$  derived from three observations per donor). Significant differences between the test compounds are indicated with different letters ( $p < 0.05$ ).

Prebiotic properties are often evaluated using changes in Bifidobacteria populations because many microbial species linked with health-promoting properties are members of this group. Bifidobacteria include many species that can degrade complex carbohydrates.<sup>38</sup> Increase in the *Bifidobacterium* spp. levels occur after oat consumption, and by extension,  $\beta$ -glucan-rich cereal.<sup>39–44</sup> Mårtensson et al.<sup>43</sup> found that supplementation with oat-based products stimulated Bifidobacteria growth in the gastrointestinal tracts of hypercholesterolemic human subjects. This change was accompanied by a reduction in plasma cholesterol levels. Queenan et al.<sup>45</sup> found that total cholesterol and low-density lipoprotein cholesterol concentrations were significantly lower in hypercholesterolemic adults after daily supplementation of oat  $\beta$ -glucans for 6 weeks. The increase in Bifidobacteria for the oat products we investigated thus confirms that these products have potential to result in specific health benefits.

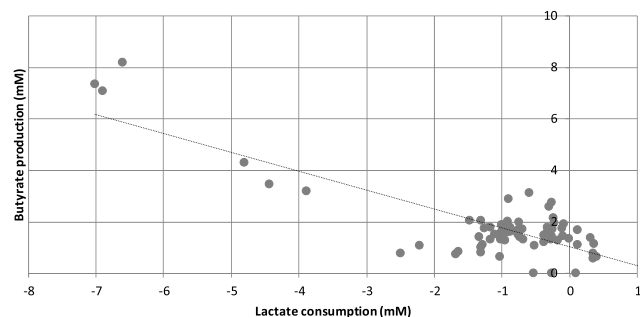
The use of oat ingredients increased the production of the metabolites, acetate and lactate, but especially propionate. In vitro<sup>24,42,46</sup> and in vivo animal<sup>47</sup> studies have revealed that selective stimulation of propionate production occurs after supplementation with oat products. The beneficial effects of oat fibers on cholesterol levels might be associated with this propionogenic effect. Propionate is transported to the liver, where it reduces cholesterol and fatty acid synthesis<sup>48–50</sup> and positively affects glycemic control.<sup>48,50</sup> Propionate also participates in the regulation of immune function in adipose tissue.<sup>51,52</sup> The stimulation of specific health-related microbial metabolites by the oat ingredients thus suggests that they might be useful as prebiotic substrates.

Oat supplementation strongly decreased branched SCFA and ammonium production. The initial production of branched SCFAs occurred between 0 and 24 h. Branched SCFAs reached maximum levels between 24 and 48 h for all oat ingredients, but ammonium was mainly produced during the first 24 h of incubation. The formation of branched SCFAs and ammonium was likely due to the depletion of the product and the subsequent shift to proteolytic fermentation. Because proteolytic fermentation results in the production of toxic compounds such as *p*-cresol,<sup>53,54</sup> highly branched SCFA and ammonium production in the colon are associated with detrimental health effects. Their reduction upon supplementation with the different oat ingredients confirms the high fermentability of the test substrates, in this way postponing proteolytic fermentation.

The results indicated that during the final 24 h of incubation there were strong correlations between lactate consumption and butyrate production in the FOS and the tested oat ingredient samples. In general, the consumption of lactate during the final 24 h was much lower for the oat ingredients compared with FOS, and the highest production of butyrate occurred with FOS supplementation. However, the non-consumed amounts of lactate that remained at the end of the incubation period was similar for all tested products, including FOS. Lactate is produced by lactic acid bacteria and decreases the pH of the intestinal environment.<sup>55</sup> Especially at low pH values, lactate can exert strong antimicrobial effects against pathogens. Protonated lactic acid can penetrate the microbial cell wall. It then dissociates and releases protons into the cytoplasm, which results in acidification and microbial cell death.<sup>56–58</sup> Because of this antipathogenic activity, lactate accumulation can be considered to be a health-promoting prebiotic effect. Another beneficial effect of lactate results from



**Figure 2.** Microbial metabolic activity (SCFA and lactate production). Mean values for (A) acetate, (B) propionate, (C) butyrate, and (D) lactate production (mM) during the initial 0–24 h and the 24–48 h time intervals, during fermentation of test compounds (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked oat flour; 6 = pre-cooked Morrison oat flour; 7 = fructooligosaccharide) by human fecal microbiota of three different donors (A, B, and C) versus their respective blank negative controls (BNC). For optimal observation of consistent overall microbiota-related effects, the mean values (donor A/B/C) are presented ( $n = 9$ ; derived from three observations per donor). Significant differences between the test compounds are indicated with different letters ( $p < 0.05$ ). For acetate (A), propionate (B) and butyrate (C), significant differences are indicated for the 0–48 h time interval, while for lactate (D) differences are indicated for both the 0–24 h and the 24–48 h time intervals.



**Figure 3.** Correlation between lactate consumption and butyrate production. Lactate consumption and butyrate production (mM) during the 24–48 h time interval, upon fermentation of test compounds (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked oat flour; 6 = pre-cooked Morrison oat flour; 7 = fructooligosaccharide) by human fecal microbiota of three different donors (A, B, and C) versus their respective blank negative controls (BNCs).

its conversion to the health-promoting SCFA butyrate.<sup>59</sup> The accumulation of lactate at the end of incubation indicated that there was a high potential for butyrate production by the oat ingredients and FOS. In vivo, accumulated lactate levels would likely be transported to the distal colon regions. Specific butyrate-producing strains of bacteria such as *Anaerostipes caccae* and *Eubacterium hallii* could then convert the lactate to butyrate.<sup>60</sup> Because many colonic diseases originate in the

distal colon,<sup>61</sup> the tested products (including FOS) could have a beneficial effect by shifting a part of fermentation to the more distal regions of the gastrointestinal tract.

The results for the tested oat ingredients revealed the stimulation of product-specific microbial pathways. The microbiome effects of old-fashioned oats, instant oat flakes, pre-cooked oat flour, and pre-cooked Morrison oat flour were similar. However, the microbial interactions that occurred after treatment with oat bran and steel-cut oats tended to be more different from the other oat ingredients. The results for the use of steel-cut oats indicated that its effects were similar to the negative control. The microbial pattern obtained when oat bran was used was more closely related to the results for FOS use. FOS stimulates the growth of *Bifidobacterium* species in the human colon.<sup>9,10</sup> Among the oat ingredients tested in this study, oat bran resulted in the highest bifidogenic effect. Oat bran originates from the aleurone layers of the oat groats and is the coarser fraction obtained after the groats are ground into flour. Oat bran is characterized by high protein and total dietary fiber content.<sup>62</sup> It had the highest levels of these components of all the oat ingredients tested in this study. Steel-cut oats are produced by cutting the whole oat groats into smaller pieces.<sup>62</sup> Mechanical processing increases the extractability of dietary fibers (e.g.,  $\beta$ -glucans) from the oat product,<sup>19</sup> so the limited processing and larger particle size of steel-cut oats could explain why the post-treatment metabolic profile was more closely related to the negative control than to the other oat ingredients. The low amount of total dietary fiber

Table 3. Microbial Metabolic Activity (Branched SCFA and  $\text{NH}_4^+$  Production)<sup>a</sup>

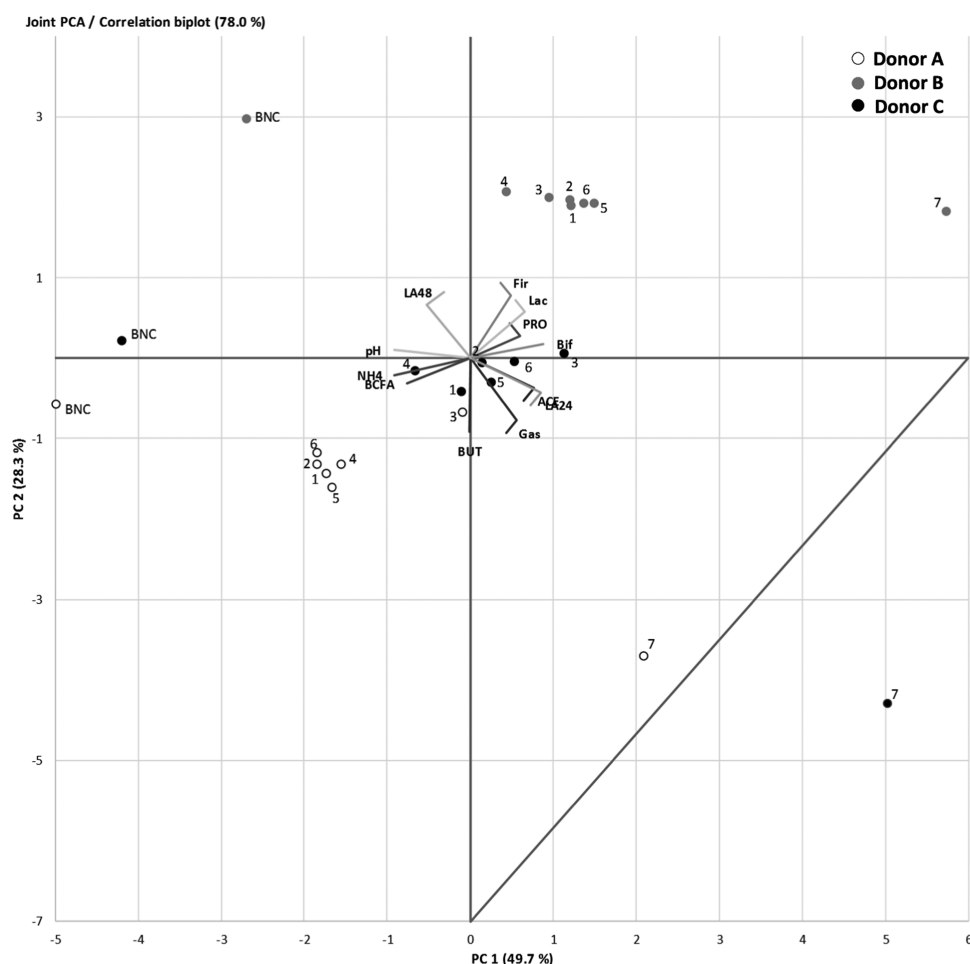
	time (h)	donor A/B/C						
		1	2	3	4	5	6	7
bSCFA (mM)	Δ0–24	0.24 <sup>ab</sup> ± 0.06	0.25 <sup>ab</sup> ± 0.08	0.19 <sup>abc</sup> ± 0.19	0.28 <sup>ab</sup> ± 0.11	0.30 <sup>a</sup> ± 0.07	0.14 <sup>bc</sup> ± 0.09	0.04 <sup>c</sup> ± 0.11
	Δ24–48	0.61 <sup>a</sup> ± 0.89	0.58 <sup>a</sup> ± 0.9	0.06 <sup>a</sup> ± 0.96	0.71 <sup>a</sup> ± 0.91	0.85 <sup>b</sup> ± 1.10	0.30 <sup>a</sup> ± 1.11	0.12 <sup>a</sup> ± 0.15
	Δ0–48	0.86 ± 0.94	0.83 ± 0.95	0.25 ± 1.05	0.99 ± 1.00	1.16 ± 1.17	0.44 ± 1.16	0.16 ± 0.25
$\text{NH}_4^+$ (mg/L)	Δ0–24	212.8 <sup>a</sup> ± 41.6	219.4 <sup>a</sup> ± 37.2	215.9 <sup>a</sup> ± 42.8	227.3 <sup>a</sup> ± 46.3	219.5 <sup>a</sup> ± 46.4	197.4 <sup>a</sup> ± 41.4	89.1 <sup>b</sup> ± 57.9
	Δ24–48	70.7 ± 28.6	66.2 ± 29.5	38.4 ± 133.1	81.2 ± 32.2	66.5 ± 22.3	81.6 ± 48.9	42.3 ± 28.5
	Δ0–48	283.4 ± 40.6	285.6 ± 47.7	254.3 ± 123.5	308.5 ± 58.1	286.0 ± 45.2	278.9 ± 48.9	131.4 ± 84.5

<sup>a</sup>Mean (±standard deviation) values for branched SCFA (mM) and  $\text{NH}_4^+$  production (mg/L) between 0–24, 24–48, and 0–48 h upon fermentation of the different test compounds (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked Morrison oat flour; 6 = pre-cooked Morrison oat flour; 7 = fructooligosaccharide) by fecal microbiota of three different human donors (A, B, and C), versus their respective blank negative controls (BNCs). For optimal observation of consistent effects over the microbiota of the three donors, the mean values (donor A/B/C) are presented ( $n = 9$ ;  $n = 3$  derived from three observations per donor). Significant differences between the test compounds are indicated with different letters ( $p < 0.05$ ).

Table 4. qPCR Results for Microbial Community Composition<sup>a</sup>

	donor	donor A/B/C						
		1	2	3	4	5	6	7
Firmicutes	A/B/C	0.08 ± 0.35	0.12 ± 0.31	0.17 ± 0.19	0.12 ± 0.29	0.11 ± 0.39	0.08 ± 0.37	0.20 ± 0.33
Bifidobacteria	A/B/C	0.52 <sup>a</sup> ± 0.30	0.61 <sup>a</sup> ± 0.32	0.93 <sup>a</sup> ± 0.40	0.60 <sup>a</sup> ± 0.16	0.65 <sup>a</sup> ± 0.30	0.59 <sup>a</sup> ± 0.31	1.03 <sup>b</sup> ± 0.19
<i>Lactobacillus</i>	A/B/C	0.61 <sup>a</sup> ± 0.96	0.70 <sup>a</sup> ± 0.95	0.71 <sup>a</sup> ± 0.70	0.63 <sup>a</sup> ± 0.91	0.74 <sup>a</sup> ± 1.05	0.70 <sup>a</sup> ± 0.88	1.84 <sup>b</sup> ± 1.50
A		−0.03 <sup>a</sup> ± 0.14	0.02 <sup>ab</sup> ± 0.09	0.33 <sup>b</sup> ± 0.25	−0.03 <sup>ab</sup> ± 0.09	−0.03 <sup>ab</sup> ± 0.13	0.00 <sup>ab</sup> ± 0.08	0.06 <sup>ab</sup> ± 0.11
B		1.89 <sup>ab</sup> ± 0.03	1.95 <sup>abc</sup> ± 0.08	1.61 <sup>ab</sup> ± 0.07	1.84 <sup>ab</sup> ± 0.05	2.13 <sup>bc</sup> ± 0.07	1.86 <sup>ab</sup> ± 0.03	3.51 <sup>c</sup> ± 0.11
C		−0.02 <sup>abc</sup> ± 0.05	0.12 <sup>bc</sup> ± 0.06	0.21 <sup>c</sup> ± 0.29	0.07 <sup>abc</sup> ± 0.02	0.11 <sup>bc</sup> ± 0.06	0.22 <sup>bc</sup> ± 0.02	1.94 <sup>bc</sup> ± 0.03

<sup>a</sup>Mean (±standard deviation) values for changes in *Firmicutes*, *Bifidobacteria*, and *Lactobacillus* (log 10 16S rRNA copies/mL) during the 0–48 h time interval upon fermentation of test compounds (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked Morrison oat flour; 6 = pre-cooked Morrison oat flour; 7 = fructooligosaccharide) by fecal microbiota of three different human donors (A, B, and C), versus their respective blank negative controls (BNCs). For optimal observation of consistent effects over the microbiota of the three donors, the mean values (donor A/B/C) are presented ( $n = 9$ ;  $n = 3$  per donor). For *Lactobacillus*, also the mean individual levels are shown as they were characterized by distinct inter-donor differences. Significant differences between the test substances are indicated with different letters ( $p < 0.05$ ).



**Figure 4.** Principal component analysis. PCA biplot obtained for the effects of the six oat ingredients on gut microbiota activity and composition, compared with a positive and a negative control. The biplot plots the variables (analytes) as vectors and the observations (test) as dots. Each observation consists of a white (donor A), gray (donor B), or black (donor C) dot and a number from 1 to 7 (or BNC), which indicate the specific test conditions (1 = old-fashioned oats; 2 = instant oat flakes; 3 = oat bran; 4 = steel-cut oats; 5 = pre-cooked oat flour; 6 = pre-cooked Morrison oat flour; 7 = fructooligosaccharide). The first two principal components explained 78% of variation.

and the highly branched SCFA and ammonium production after treatment with steel-cut oats indicated a faster depletion of the product with an associated shift to proteolytic fermentation<sup>53,54</sup> and confirms this conclusion. Therefore, the results suggested that there was an association between the total amount of production process-related dietary fiber present in different oat ingredients and the prebiotic potential of each ingredient.

Interpretation of the microbial interactions that occurred after treatment with different oat ingredients indicated that these interactions were also donor-specific. Overall, the results for donor A were characterized by a high initial fermentation rate and gas production (0–6 h) for FOS and all oat ingredients. Donors B and C had lower fermentation and gas production rates. This result was confirmed by the high initial propionate levels (0–6 h) for donor A, compared with donor C and especially donor B. These differences indicated that the donors had different primary substrate degraders. Propionate can be produced by a wide range of gut microbes; *Bacteroides* spp.<sup>63,64</sup> and *Akkermansia muciniphila*<sup>65</sup> are the most prevalent of the gut species that produce propionate. *A. muciniphila* is a mucin-degrading bacterium,<sup>65</sup> while bacteria belonging to the *Bacteroidetes* phylum, are known for their fiber-degrading potential.<sup>66</sup> The strong initial propionate production for donor

A suggested that propionate producers were relatively more prevalent in the starting fecal community of this donor. The interindividual differences in microbial composition were also indicated by the *Lactobacillus* spp. levels. Donor B had very low initial levels, compared with donors A and C. Taken together, the results indicated that although the overall post-treatment microbial metabolism was similar between different donors, the differences in microbial community composition affected the use of the oat ingredients in a donor-specific manner.

In conclusion, the use of different oat ingredients revealed that there was stimulation of health-related microbial metabolites in the gut microbiome. This result suggested that these ingredients might be used as prebiotic substrates upon digestion. Overall, the levels of health-related metabolites and *Bifidobacterium* spp. increased after treatment, but the different products were associated with the stimulation of specific metabolic pathways and changes in the composition of the gut microbiome. The results also suggested that there was an association between the total amount of production process-related dietary fiber present in different oat ingredients and the prebiotic potential of each ingredient.



## MATERIALS AND METHODS

**Chemicals and Carbohydrates.** All chemicals were obtained from Sigma-Aldrich (Overijse, Belgium) unless indicated otherwise. PepsiCo, Inc. (Barrington) provided different oat ingredients, which were produced by the application of different processing techniques (Table 1). All oat ingredients started off as oat groats. Groats were roasted at low temperature (80–100 °C) for stabilization before further process. To obtain steel-cut oats, whole groats were cut into several pieces. Due to the large particle size, these steel-cut oats take the longest to cook. Old-fashioned oats were produced by steaming the whole groats to make them soft and pliable, and then pressed to flatten them obtaining a particle size of 0.51–0.76 mm. Furthermore, steel-cut oats were used for the preparation of instant oat flakes. They were heat-treated using steam and pressed slightly thinner than old-fashioned oats obtaining a particle size of 0.36–0.46 mm. Instant oat flakes were ground to smaller particle size and fine particles of oat flour were removed using a sieving process yielding oat bran. Pre-cooked oat flour was produced using PepsiCo proprietary process providing ready-to-use format with improved dispersability. Granulation range for the pre-cooked oat flour ranged from 50 to 250  $\mu\text{m}$  with targeting of 178–250  $\mu\text{m}$ . All previously mentioned oat ingredients started from the same lot of oats. Morrison oat is a PepsiCo proprietary variety containing higher  $\beta$ -glucan and protein than regular oat varieties. The Morrison oat flour was processed in the same way as pre-cooked oat flour.

The positive control for the study was FOS with a purity of 99%, and a degree of polymerization >10.

**In Vitro Digestion Experiments for Oat Ingredients and FOS.** Digestion experiments were performed to produce relevant product fractions of the six oat ingredients (Table 1) and that would also reach the colon. Blank experiments were performed for two remaining conditions (i.e., blank control (BNC) and FOS). The test ingredients were prepared according to the manufacturers' instructions (Table 1) and then were subjected to oral, gastric, and small intestinal incubation. Incubation conditions were based on the consensus digestion protocol, which was developed within the large European-framework COST Action Infogest, with some improvements. The oral phase was implemented as proposed by Mackie et al.,<sup>33</sup> but at the beginning of gastric incubation, the ingredients were diluted to obtain a concentration of 80 g DW/L in the stomach and 56 g DW/L in the small intestine, corrected for the moisture content after cooking. The enzyme versus product ratios was maintained as proposed by the consensus method. Working with more dilute aqueous matrices allowed improvement in the protocol; a pH profile from 5.5 to 2.0 during gastric incubation was used to mimic in vivo fed gastric conditions more closely. A dialysis approach (validated for glucose and amino acids (data not shown)) was used for the simulation of small intestinal absorption, and removal of small molecules (<3.5 kDa) from the intestinal digests. Dialysis was started after 30 min of small intestinal incubation. The intestinal digest was added to a dialysis tube (ZelluTrans/Roth dialysis membrane, regenerated cellulose, molecular weight cut off 3.5 kDa), which was submerged in double the volume of dialysis fluid (3.75 g/L  $\text{NaHCO}_3$ ; pH 7.0). During the 3 h incubation, the dialysis fluid was replaced once each hour with fresh fluid. This dialysis

step was not included for the low molecular weight FOS samples because it would have been lost during the procedure.

Next, colonic incubation was initiated by adding 12.5 mL of the digested and dialyzed liquid from the small intestine to 50.5 mL colonic background medium (4.45 g/L  $\text{K}_2\text{HPO}_4$ ; 13.6 g/L  $\text{KH}_2\text{PO}_4$ ; 2.5 g/L  $\text{NaHCO}_3$ ; 2.5 g/L yeast extract; 2.5 g/L peptone; 1.25 g/L mucin; 0.625 g/L L-cysteine HCl; 2.5 mL/L Tween 80) in 120 mL volume penicillin bottles. When a product would entirely escape upper gastrointestinal tract digestion and absorption, adding 12.5 mL of intestinal digests would correspond to a maximum concentration of 10 g/L. When digestion and absorption is taken into account, this would correspond to a dose of approximately 5 g/L. A total of 5 g FOS/L was added as a positive control. Anaerobiosis was obtained using  $\text{N}_2$  gas and alternation between vacuum and overpressure conditions for 10 cycles. Each human fecal inoculum that was subsequently prepared from each of the three healthy volunteers (donor A = male, 30 years; donor B = male, 29 years; donor C = male, 32 years) was a 1:13 (mass/volume) mixture of a freshly collected fecal sample and anaerobic phosphate buffer ( $\text{K}_2\text{HPO}_4$  8.8 g/L;  $\text{KH}_2\text{PO}_4$  6.8 g/L; sodium thioglycolate 0.1 g/L; sodium dithionite 0.015 g/L). After homogenization (10 min, BagMixer 400, Interscience, Louvain-La-Neuve, Belgium) and removal of large particles via centrifugation (2 min, 500g), 7 mL inoculum was added to the different bottles. The samples were then incubated for 48 h at 37 °C, and under 90 rpm agitation conditions. Each experiment was performed in triplicate for each donor and each treatment. As a remark, the human volunteers included in the study were considered as healthy adults with a body mass index between 20 and 25, following a general westernized diet and did not take any antibiotic treatment for at least 6 months prior to fecal donation.

**Starch Determination Method.** Total starch quantification was performed using a commercially available enzymatic assay kit (Megazyme, Wicklow, Ireland) according to the manufacturer's instructions. Briefly, starch was converted to maltodextrins via thermostable  $\alpha$ -amylase at 100 °C; KOH and  $\alpha$ -amylase were used for the conversion of resistant starch. The maltodextrins were then hydrolyzed to D-glucose by amyloglucosidase. D-Glucose was oxidized to D-gluconate, and the released hydrogen peroxide was measured colorimetrically using peroxidase and the production of a quinoneimine dye. Quantification of starch levels in the initial test ingredients and at the end of the small intestinal incubation allowed for the calculation of digestible and nondigestible starch fractions.

**Microbial Metabolic Activity Analysis.** The pH (Sense-line F410; ProSense, Oosterhout, The Netherlands), gas (hand-held pressure indicator CPH6200; Wika, Echt, The Netherlands), lactate (LA), ammonium ( $\text{NH}_4^+$ ), and short-chain fatty acid (SCFA) measurements were performed at 0, 6, 24, and 48 h after starting the colonic incubation. Acetate, propionate, butyrate, and branched SCFAs (isobutyrate, isovalerate, and isocaproate) were measured as described by De Weirtdt et al.<sup>34</sup> Lactate quantification was performed using a commercially available enzymatic assay kit (R-Biopharm, Darmstadt, Germany) according to the manufacturer's instructions. Ammonium analysis was performed as described by Van de Wiele et al.<sup>35</sup>

**Microbial Community Analysis.** At 0 and 48 h, the samples were collected for microbial community analysis. Total DNA was extracted as described by Boon et al.,<sup>36</sup> with some



minor modifications. The DNA was extracted from a pellet of bacterial cells originated from a 1 mL sample after centrifugation for 5 min at 7700g. A Fastprep-24 device (MP BioMedicals, Illkirch, France) was used for homogenization, which was performed twice for 40 s at 4 m/s; the sample was allowed to rest for 5 min between shakings. Subsequently, the quantitative PCR (qPCR) assays for *Firmicutes*, *Lactobacillus* spp., and *Bifidobacterium* spp. were performed using a StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA). Each sample was analyzed in technical triplicate and outliers (more than 1  $C_T$  difference) were omitted. The samples were checked for correct melt curve peaks. The standard curves for all of the different runs had efficiencies between 90 and 105%. Descriptions of the primers, temperature conditions, and primer concentrations are presented in Supporting Information Table 1.

**Statistical Analysis.** The statistical analyses were performed using Statistical Analysis Software version 9.4 (SAS Institute: 191 Cary, NC) and Minitab 17 Statistical Software (Minitab Inc; State College, PA). A two-way analysis of variance (factors were oat ingredient type and donor) was performed to investigate the effects of different oat ingredients on microbial metabolic activities. Tukey's test was used for post hoc analysis. The significance level for all statistical tests was  $\alpha = 0.05$ .

Principal component analysis was performed using Analyze-it (v4.51) software. The increase or decrease from 0 to 48 h of incubation for each parameter (except for lactate, which was split into two parameters, i.e., 0–24 h for production and 24–48 h for consumption) was used to create a joint PCA biplot.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01360.

Primers and cycling programs used for qPCR quantification of species-specific 16S rDNA (Table 1); overall metabolic activity (acidification and gas production) (Table 2); interindividual variability in microbial metabolic activity parameters (Table 3); microbial metabolic activity (SCFA and lactate production) (Table 4); microbial metabolic activity (branched SCFA and  $\text{NH}_4^+$  production) (Table 5) (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: [massimo.marzorati@prodigest.eu](mailto:massimo.marzorati@prodigest.eu). Phone: +3292411190. Fax: +3292411191.

### ORCID

Massimo Marzorati: 0000-0003-4955-180X

### Notes

The authors declare the following competing financial interest(s): A.K., L.F., Y.C., and P.D.C. are employees from PepsiCo, Inc.

The views expressed in this report are those of the authors and do not necessarily represent the position or policy of PepsiCo, Inc.

## ■ ACKNOWLEDGMENTS

M.M. is a postdoctoral fellow from FWO-Vlaanderen (Research Foundation of Flanders, Belgium). Jonas Ghyselink and Cindy Duysburgh from ProDigest bvba were compensated for providing technical assistance and writing assistance, respectively.

## ■ ABBREVIATIONS

BNC, blank negative control  
FOS, fructooligosaccharide  
LA, lactate  
 $\text{NH}_4^+$ , ammonium  
qPCR, quantitative PCR  
SCFA, short-chain fatty acid

## ■ REFERENCES

- (1) Salminen, S.; Bouley, C.; Boutron, M. C.; Cummings, J. H.; Franck, A.; Gibson, G. R.; Isolauri, E.; Moreau, M. C.; Roberfroid, M.; Rowland, I. Functional food science and gastrointestinal physiology and function. *Br. J. Nutr.* **1998**, *80*, S147–S171.
- (2) Delzenne, N. M.; Williams, C. M. Prebiotics and lipid metabolism. *Curr. Opin. Lipidol.* **2002**, *13*, 61–67.
- (3) Bäckhed, F.; Ding, H.; Wang, T.; Hooper, L. V.; Koh, G. Y.; Nagy, A.; Semenkovich, C. F.; Gordon, J. I. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15718–15723.
- (4) Possemiers, S.; Bolca, S.; Grootaert, C.; Heyerick, A.; Decroos, K.; Dhooze, W.; De Keukeleire, D.; Rabot, S.; Verstraete, W.; Van de Wiele, T. The prenylflavonoid isoxanthohumol from hops (*Humulus lupulus* L.) is activated into the potent phytoestrogen 8-prenylnaringenin in vitro and in the human intestine. *J. Nutr.* **2006**, *136*, 1862–1867.
- (5) Hughes, R.; Rowland, I. R. Metabolic Activities of the Gut Microflora in Relation to Cancer. *Microb. Ecol. Health Dis.* **2000**, *12*, 179–185.
- (6) Lupton, J. R. Microbial degradation products influence colon cancer risk: the butyrate controversy. *J. Nutr.* **2004**, *134*, 479–482.
- (7) Gibson, G. R.; Hutkins, R.; Sanders, M. E.; Prescott, S. L.; Reimer, R. A.; Salminen, S. J.; Scott, K.; Stanton, C.; Swanson, K. S.; Cani, P. D.; Verbeke, K.; Reid, G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 491.
- (8) Gibson, G. R.; Roberfroid, M. B. *Handbook of Prebiotics*; CRC Press: Boca Raton, FL, 2008; p 504.
- (9) Sivieri, K.; Morales, M. L.; Saad, S. M.; Adorno, M. A.; Sakamoto, I. K.; Rossi, E. A. Prebiotic effect of fructooligosaccharide in the simulator of the human intestinal microbial ecosystem (SHIME(R) model). *J. Med. Food* **2014**, *17*, 894–901.
- (10) Gibson, G. R.; Beatty, E. R.; Wang, X.; Cummings, J. H. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **1995**, *108*, 975–982.
- (11) Braaten, J. T.; Wood, P. J.; Scott, F. W.; Wolynetz, M. S.; Lowe, M. K.; Bradley-White, P.; Collins, M. W. Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* **1994**, *48*, 465–474.
- (12) Bae, I. Y.; Kim, S. M.; Lee, S.; Lee, H. G. Effect of enzymatic hydrolysis on cholesterol-lowering activity of oat beta-glucan. *New Biotechnol.* **2010**, *27*, 85–88.
- (13) Tiwari, U.; Cummins, E. Meta-analysis of the effect of beta-glucan intake on blood cholesterol and glucose levels. *Nutrition* **2011**, *27*, 1008–1016.
- (14) Wood, P. J.; Beer, M. U.; Butler, G. Evaluation of role of concentration and molecular weight of oat beta-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. *Br. J. Nutr.* **2000**, *84*, 19–23.

- (15) Hooda, S.; Matte, J. J.; Vasanthan, T.; Zijlstra, R. T. Dietary purified oat  $\beta$ -glucan reduces peak glucose absorption and portal insulin release in portal-vein catheterized grower pigs. *Livest. Sci.* **2010**, *134*, 15–17.
- (16) El Khoury, D.; Cuda, C.; Luhovyy, B. L.; Anderson, G. H. Beta Glucan: Health Benefits in Obesity and Metabolic Syndrome. *J. Nutr. Metab.* **2012**, *2012*, No. 851362.
- (17) Daou, C.; Zhang, H. Oat Beta-Glucan: Its Role in Health Promotion and Prevention of Diseases. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11*, 355–365.
- (18) Volman, J. J.; Ramakers, J. D.; Plat, J. Dietary modulation of immune function by beta-glucans. *Physiol. Behav.* **2008**, *94*, 276–284.
- (19) Grundy, M. M.-L.; Quint, J.; Rieder, A.; Ballance, S.; Dreiss, C. A.; Butterworth, P. J.; Ellis, P. R. Impact of hydrothermal and mechanical processing on dissolution kinetics and rheology of oat  $\beta$ -glucan. *Carbohydr. Polym.* **2017**, *166*, 387–397.
- (20) Zhang, D.; Doehlert, D. C.; Moore, W. R. Rheological Properties of (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-Glucans from Raw, Roasted, and Steamed Oat Groats. *Cereal Chem.* **1998**, *75*, 433–438.
- (21) Regand, A.; Chowdhury, Z.; Tosh, S. M.; Wolever, T. M. S.; Wood, P. The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. *Food Chem.* **2011**, *129*, 297–304.
- (22) Anttila, H.; Sontag-Strohm, T.; Salovaara, H. Viscosity of beta-glucan in oat products. *Agric. Food Sci.* **2004**, *13*, 80–87.
- (23) Kaur, A.; Rose, D. J.; Rumpagaporn, P.; Patterson, J. A.; Hamaker, B. R. In vitro batch fecal fermentation comparison of gas and short-chain fatty acid production using “slowly fermentable” dietary fibers. *J. Food Sci.* **2011**, *76*, H137–H142.
- (24) Hughes, S. A.; Shewry, P. R.; Gibson, G. R.; McCleary, B. V.; Rastall, R. A. In vitro fermentation of oat and barley derived beta-glucans by human faecal microbiota. *FEMS Microbiol. Ecol.* **2008**, *64*, 482–493.
- (25) Rycroft, C. E.; Jones, M. R.; Gibson, G. R.; Rastall, R. A. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *J. Appl. Microbiol.* **2001**, *91*, 878–887.
- (26) Allison, C.; McFarlan, C.; MacFarlane, G. T. Studies on mixed populations of human intestinal bacteria grown in single-stage and multistage continuous culture systems. *Appl. Environ. Microbiol.* **1989**, *55*, 672–678.
- (27) Minekus, M.; Smeets-Peters, M.; Bernalier, A.; Marol-Bonnin, S.; Havenaar, R.; Marteau, P.; Alric, M.; Fonty, G.; Huis in't Veld, J. H. A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl. Microbiol. Biotechnol.* **1999**, *53*, 108–114.
- (28) Molly, K.; Vande Woestyne, M.; Verstraete, W. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl. Microbiol. Biotechnol.* **1993**, *39*, 254–258.
- (29) Grootaert, C.; Van den Abbeele, P.; Marzorati, M.; Broekaert, W. F.; Courtin, C. M.; Delcour, J. A.; Verstraete, W.; Van de Wiele, T. Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiol. Ecol.* **2009**, *69*, 231–242.
- (30) Eckburg, P. B.; Bik, E. M.; Bernstein, C. N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S. R.; Nelson, K. E.; Relman, D. A. Diversity of the Human Intestinal Microbial Flora. *Science* **2005**, *308*, 1635–1638.
- (31) Turnbaugh, P. J.; Ridaura, V. K.; Faith, J. J.; Rey, F. E.; Knight, R.; Gordon, J. I. The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. *Sci. Transl. Med.* **2009**, *1*, No. 6ra14.
- (32) Conlon, M. A.; Bird, A. R. The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. *Nutrients* **2015**, *7*, 17.
- (33) Mackie, A.; Rigby, N. InfoGest Consensus Method. In *The Impact of Food Bioactives on Health: in Vitro and Ex Vivo Models*; Verhoeckx, K.; Cotter, P.; López-Expósito, I.; Kleiveland, C.; Lea, T.; Mackie, A.; Requena, T.; Swiatecka, D.; Wichers, H., Eds.; Springer International Publishing: Cham, 2015; pp 13–22.
- (34) De Weirtdt, R.; Possemiers, S.; Vermeulen, G.; Moerdijk-Poortvliet, T. C. W.; Boschker, H. T. S.; Verstraete, W.; Van de Wiele, T. Human faecal microbiota display variable patterns of glycerol metabolism. *FEMS Microbiol. Ecol.* **2010**, *74*, 601–611.
- (35) Van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H.; Verstraete, W. Prebiotic effects of chicory inulin in the simulator of the human intestinal microbial ecosystem. *FEMS Microbiol. Ecol.* **2004**, *51*, 143–153.
- (36) Boon, N.; Top, E. M.; Verstraete, W.; Siciliano, S. D. Bioaugmentation as a Tool To Protect the Structure and Function of an Activated-Sludge Microbial Community against a 3-Chloroaniline Shock Load. *Appl. Environ. Microbiol.* **2003**, *69*, 1511–1520.
- (37) Kim, H. J.; White, P. J. In vitro digestion rate and estimated glycemic index of oat flours from typical and high beta-glucan oat lines. *J. Agric. Food Chem.* **2012**, *60*, 5237–5242.
- (38) Pokusaeva, K.; Fitzgerald, G. F.; van Sinderen, D. Carbohydrate metabolism in Bifidobacteria. *Genes Nutr.* **2011**, *6*, 285–306.
- (39) Gyawali, R.; Minor, R. C.; Donovan, B.; Ibrahim, S. A. Inclusion of Oat in Feeding Can Increase the Potential Probiotic Bifidobacteria in Sow Milk. *Animals* **2015**, *5*, 610–623.
- (40) Metzler-Zebeli, B. U.; Zijlstra, R. T.; Mosenthin, R.; Gänzle, M. G. Dietary calcium phosphate content and oat  $\beta$ -glucan influence gastrointestinal microbiota, butyrate-producing bacteria and butyrate fermentation in weaned pigs. *FEMS Microbiol. Ecol.* **2011**, *75*, 402–413.
- (41) Shen, R.-L.; Dang, X.-Y.; Dong, J.-L.; Hu, X.-Z. Effects of Oat  $\beta$ -Glucan and Barley  $\beta$ -Glucan on Fecal Characteristics, Intestinal Microflora, and Intestinal Bacterial Metabolites in Rats. *J. Agric. Food Chem.* **2012**, *60*, 11301–11308.
- (42) Kedia, G.; Vázquez, J. A.; Charalampopoulos, D.; Pandiella, S. S. In Vitro Fermentation of Oat Bran Obtained by Debranning with a Mixed Culture of Human Fecal Bacteria. *Curr. Microbiol.* **2009**, *58*, 338–342.
- (43) Mårtensson, O.; Björklund, M.; Lambo, A. M.; Dueñas-Chasco, M.; Irastorza, A.; Holst, O.; Norin, E.; Welling, G.; Öste, R.; Önnings, G. Fermented, rosy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. *Nutr. Res.* **2005**, *25*, 429–442.
- (44) Zhao, J.; Cheung, P. C. K. Fermentation of  $\beta$ -Glucans Derived from Different Sources by Bifidobacteria: Evaluation of Their Bifidogenic Effect. *J. Agric. Food Chem.* **2011**, *59*, 5986–5992.
- (45) Queenan, K. M.; Stewart, M. L.; Smith, K. N.; Thomas, W.; Fulcher, R. G.; Slavin, J. L. Concentrated oat  $\beta$ -glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. *Nutr. J.* **2007**, *6*, No. 6.
- (46) Monsma, D. J.; Thorsen, P. T.; Vollendorf, N. W.; Crenshaw, T. D.; Marlett, J. A. In Vitro Fermentation of Swine Ileal Digesta Containing Oat Bran Dietary Fiber by Rat Cecal Inocula Adapted to the Test Fiber Increases Propionate Production But Fermentation of Wheat Bran Ileal Digesta Does Not Produce More Butyrate. *J. Nutr.* **2000**, *130*, 585–593.
- (47) Jackson, K. A.; Topping, D. L. Prevention of coprophagy does not alter the hypocholesterolaemic effects of oat bran in the rat. *Br. J. Nutr.* **1993**, *70*, 211–219.
- (48) Berggren, A. M.; Nyman, E. M. G. L.; Lundquist, I.; Björck, I. M. E. Influence of orally and rectally administered propionate on cholesterol and glucose metabolism in obese rats. *Br. J. Nutr.* **1996**, *76*, 287–294.
- (49) Lin, Y.; Vonk, R. J.; Slooff, M. J. H.; Kuipers, F.; Smit, M. J. Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. *Br. J. Nutr.* **1995**, *74*, 197–207.
- (50) Wong, J. M.; de Souza, R.; Kendall, C. W.; Emam, A.; Jenkins, D. J. Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* **2006**, *40*, 235–243.
- (51) Al-Lahham, S. H.; Roelofs, H.; Priebe, M.; Weening, D.; Dijkstra, M.; Hoek, A.; Rezaee, F.; Venema, K.; Vonk, R. J. Regulation

of adipokine production in human adipose tissue by propionic acid. *Eur. J. Clin. Invest.* **2010**, *40*, 401–407.

(52) Al-Lahham, S.; Roelofsens, H.; Rezaee, F.; Weening, D.; Hoek, A.; Vonk, R.; Venema, K. Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur. J. Clin. Invest.* **2012**, *42*, 357–364.

(53) Cummings, J. H.; Macfarlane, G. T. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* **1991**, *70*, 443–459.

(54) Van Craeyveld, V.; Swennen, K.; Dornez, E.; Van de Wiele, T.; Marzorati, M.; Verstraete, W.; Delaet, Y.; Onagbesan, O.; Decuyper, E.; Buyse, J.; De Ketelaere, B.; Broekaert, W. F.; Delcour, J. A.; Courtin, C. M. Structurally different wheat-derived arabinoxylooligosaccharides have different prebiotic and fermentation properties in rats. *J. Nutr.* **2008**, *138*, 2348–2355.

(55) Macfarlane, G. T.; Englyst, H. N. Starch utilization by the human large intestinal microflora. *J. Appl. Bacteriol.* **1986**, *60*, 195–201.

(56) Alakomi, H. L.; Skyttä, E.; Saarela, M.; Mattila-Sandholm, T.; Latva-Kala, K.; Helander, I. M. Lactic Acid Permeabilizes Gram-Negative Bacteria by Disrupting the Outer Membrane. *Appl. Environ. Microbiol.* **2000**, *66*, 2001–2005.

(57) Raybaudi-Massilia, R. M.; Mosqueda-Melgar, J.; Soliva-Fortuny, R.; Martín-Belloso, O. Control of Pathogenic and Spoilage Microorganisms in Fresh-cut Fruits and Fruit Juices by Traditional and Alternative Natural Antimicrobials. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 157–180.

(58) Stratford, M.; Eklund, T. Organic Acids and Esters. In *Food Preservatives*; Russell, N. J.; Gould, G. W., Eds.; Springer: Boston, MA, 2003; pp 48–84.

(59) Bourriaud, C.; Robins, R. J.; Martin, L.; Kozlowski, F.; Tenailleau, E.; Cherbut, C.; Michel, C. Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J. Appl. Microbiol.* **2005**, *99*, 201–212.

(60) Duncan, S. H.; Louis, P.; Flint, H. J. Lactate-Utilizing Bacteria, Isolated from Human Feces, That Produce Butyrate as a Major Fermentation Product. *Appl. Environ. Microbiol.* **2004**, *70*, 5810–5817.

(61) Jackson-Thompson, J.; Ahmed, F.; German, R. R.; Lai, S.-M.; Friedman, C. Descriptive epidemiology of colorectal cancer in the United States, 1998–2001. *Cancer* **2006**, *107*, 1103–1111.

(62) Decker, E. A.; Rose, D. J.; Stewart, D. Processing of oats and the impact of processing operations on nutrition and health benefits. *Br. J. Nutr.* **2014**, *112*, S58–S64.

(63) Macy, J. M.; Probst, I. The biology of gastrointestinal bacteroides. *Annu. Rev. Microbiol.* **1979**, *33*, 561–594.

(64) Hosseini, E.; Grootaert, C.; Verstraete, W.; Van de Wiele, T. Propionate as a health-promoting microbial metabolite in the human gut. *Nutr. Rev.* **2011**, *69*, 245–258.

(65) Derrien, M.; Vaughan, E. E.; Plugge, C. M.; de Vos, W. M. *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 1469–1476.

(66) Flint, H. J.; Scott, K. P.; Duncan, S. H.; Louis, P.; Forano, E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* **2012**, *3*, 289–306.